

August 16.—A growth consisting of several very small, round, white colonies appeared after three days. A stained preparation from one of these showed that they were composed of organisms resembling *Micrococcus melitensis*. Sub-cultures made.

September 29.—The growth from two agar tubes was made into an emulsion with salt solution, and an agglutination test made with serum from a rabbit immunised against *Micrococcus melitensis*, Malta strain.

The result was that the organism from the goats agglutinated completely in a dilution of 1 in 100, and the proof was complete that the Ankole goats are liable to contract Malta fever, and to act as a reservoir of the virus.

#### *Conclusions.*

1. "Muhinyo" is Malta fever.
  2. "Muhinyo" is conveyed from the goat to man by the drinking of goats' milk.
- 

#### *The Natural Food of Glossina palpalis.*

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908-10.)

(Received June 15,—Read June 30, 1910.)

As a good deal of interest, and it may be importance, attaches to the food of the tsetse fly—the carrier of Sleeping Sickness—the following notes are placed on record :—

In the laboratory it was found that the flies fed with far more avidity on birds than on monkeys, while they could hardly be tempted to feed on young crocodiles, iguanas, or lizards. It was very marked, this preference of theirs for birds; the moment a chicken was placed against the netting of the cage, they instantly swarmed on it in hundreds. From this it was thought probable that the natural food of the flies would prove to be birds' blood, but the two following experiments do not bear this out to any great extent :—

The first experiment was carried out in the laboratory at Mpumu, and extended over several months. Flies which had been caught on the Lake-shore and which had been kept over from the previous day, and sometimes longer, were dissected, and each portion of the alimentary canal examined in the fresh state under a low power. The various organs of the fly were then

smeared separately, and after staining (with one of the modifications of the Romanowsky stain) were examined in the usual way.

The dissections were carried out as follows:—The pharynx and proboscis were removed with a needle, and having been placed under a cover-glass and slightly opened out by pressure were examined in the fresh state. The tube of the proboscis was in this way admirably displayed, and the presence of red blood corpuscles or of flagellates could readily be determined. The alimentary canal was then removed from the abdomen, and the salivary glands detached from the fat-body. The alimentary canal was then unravelled and laid out in its full length on a slide. The whole of the tube could then be passed in review, from the junction of the thoracic intestine with the fore-gut down to the rectum, and the contents at various levels determined, as far as possible, with medium power magnifications. The main portions of the alimentary canal were then noted, and these were separated from one another by cross cuts with a sharp-edged dissecting needle. In this way the fore-gut, mid-gut, hind-gut, and proctodæum were separately removed and smeared on a slide. The smears (whilst still moist) were exposed to the vapour of osmic acid for a few seconds, and then passed through alcohol before washing and staining. The proventriculus can, with a little practice, be removed intact from the ventral aspect of the thorax. If necessary, the thoracic portions of the salivary ducts can also be recovered.

Speaking in general terms, the contents of the various portions of the alimentary canal could be determined with considerable accuracy from the naked-eye appearance during dissection. If the fly has fed very recently, the blood oozes from its proboscis when it is handled. The proventriculus, the crop or sucking stomach, as well as the fore-gut, are greatly distended with red blood. When the fed fly is put aside the blood-cells generally disappear from the proboscis in a few hours, though they may exceptionally be found as long as 24 hours after a feed. Similarly, the proventriculus and thoracic intestine quickly empty themselves, and the crop discharges its contents into the upper alimentary canal within 24 hours.

The blood travels slowly down the alimentary canal, changing in appearance as it goes. The outline of the red cells can sometimes be distinguished three days after a feed, but only when they have been retained in the fore-gut. When the blood reaches the mid-gut it begins to disintegrate and becomes homogeneous and somewhat translucent; when it reaches the lower part of the mid-gut it first becomes dark and eventually black and tarry. When in this state all cell elements are lost, and the colouring matter of the red cells is recognisable in the form of amorphous black granules. When

the contents pass posterior to the Malpighian tubes, that is, into the hind-gut, they at once become faecal in character, and turn into a dirty, yellowish-brown material, which is microscopically composed of fine granules. It is passed from the fly in this form.

The figures given in the subjoined table refer to flies which were caught at various parts of the Lake-shore, and were generally kept for about 24 hours before dissection. The majority of the dissections were done during October, November, and December, 1908.

Table I.—The Contents of the Alimentary Canal of 220 *Glossina palpalis*.

Total flies examined.	Intestine empty.	Intestine contained blood.	Character of Blood.		
			Mammalian.	Non-mammalian.	Non-recognisable.
220	160	60	20	9	31
Percentages...	72·7	27·3	9·1	4·1	14·1
			27·3		

*Remarks.*—In two cases blood corpuscles were recognised as being derived from monkeys, as the characteristic parasites of monkey malaria were found in them.

The second experiment to ascertain the food supply of *Glossina palpalis* was modified as follows:—

A journey was made to a small peninsula, hereafter referred to as “Crocodile Point.” This place was distant about two and a-half to three hours by canoe from the Kibanga landing-place, and lay in a sheltered bay far out of the beaten track of the canoes which come backwards and forwards between the islands of Kome, Damba, and Buvuma, and the weekly market at Kibanga clearing. It was chosen, therefore, partly because it was isolated from human influences, and also because of the large number of flies which lived there and the number of crocodiles and birds which frequented it.

When it was first visited, a large crocodile was disturbed from where she was lying outstretched on a spit of sand. The canoe-men at once set to work, and unearthed 58 crocodile eggs lying in layers about 18 inches below the surface, over which the “form” of the parent was clearly defined in the soil.

The peninsula was pointed in shape, and not more than 60 yards long, and was closed on the land side by the dense wall of forest which fringes

the Lake-shore. It was scattered with light undergrowth and fringed by ambatch trees, on which flocks of divers and cormorants sat with outstretched wings drying in the sun. A barrier of bare rocks and boulders projected on one side, and a small school of hippopotami was generally to be seen floating near and basking in the sun. Many small crocodiles were disturbed from the undergrowth as the point was explored, and various kinds of small land birds frequented the reeds and shrubs. The soil was sandy loam, and shaded by the light foliage. *Glossina palpalis* swarmed. The place has been described at some length, so that the exact conditions may be realised.

Subsequent to this experiment, the daily catch of Lake-shore flies was obtained from this place, and incidentally it may be added that the flies caught here were regularly found to be infective to monkeys.

Table II.—The Naked-Eye Appearances of the Contents of the Alimentary Canal of 183 Tsetse Flies, with their Sex, and the Presence or Absence of a Larva.

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
1	♂	—	—	—	34	♂	—	—	—
2	♂	—	—	—	35	♀	—	+	—
3	♂	—	—	—	36	♀	—	—	+
4	♀	+	—	—	37	♀	+	—	—
5	♀	—	—	—	38	♂	—	+	—
6	♂	—	—	—	39	♀	—	—	+
7	♀	—	—	—	40	♀	—	—	+
8	♀	+	—	—	41	♂	—	—	+
9	♂	—	—	—	42	♂	—	+	—
10	♀	—	—	—	43	♂	—	—	+
11	♀	—	—	—	44	♀	+	—	—
12	♀	—	—	—	45	♀	—	—	+
13	♀	—	—	—	46	♂	—	—	+
14	♂	—	—	—	47	♀	—	—	+
15	♂	—	—	—	48	♀	—	—	+
16	♂	—	—	—	49	♂	—	—	+
17	♀	—	—	+	50	♂	—	—	—
18	♀	—	—	+	51	♂	—	—	+
19	♀	—	+	—	52	♀	+	—	—
20	♀	—	—	+	53	♂	—	—	—
21	♂	—	+	—	54	♀	—	+	+
22	♂	—	—	+	55	♂	—	—	—
23	♀	—	—	+	56	♀	—	—	+
24	♀	—	—	+	57	♂	—	—	—
25	♂	—	+	—	58	♀	+	—	—
26	♂	—	—	+	59	♀	—	—	+
27	♂	—	+	+	60	♀	—	—	+
28	♀	+	—	+	61	♂	—	—	+
29	♂	—	—	+	62	♂	—	—	+
30	♂	—	—	—	63	♂	—	—	+
31	♀	—	—	+	64	♀	—	—	—
32	♀	+	+	—	65	♂	—	—	—
33	♀	—	—	+	66	♂	—	—	+

Table II—*continued*.

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
67	♂	—	—	—	126	♂	—	—	+
68	♂	—	—	—	127	♂	—	—	—
69	♂	—	—	—	128	♂	—	—	+
70	♂	—	—	—	129	♂	—	—	+
71	♂	—	+	—	130	♂	—	—	+
72	♂	—	—	+	131	♂	—	+	+
73	♂	—	—	—	132	♂	—	+	+
74	♂	+	+	+	133	♂	—	+	+
75	♂	—	+	+	134	♂	—	+	+
76	♂	—	—	+	135	♂	—	—	—
77	♂	—	+	+	136	♂	—	—	—
78	♂	—	+	—	137	♂	+	—	+
79	♂	—	—	—	138	♂	—	—	+
80	♂	—	—	—	139	♂	—	—	—
81	♂	—	—	—	140	♂	—	—	+
82	♂	—	+	+	141	♂	—	—	+
83	♂	+	—	—	142	♂	—	—	+
84	♂	+	—	—	143	♂	—	+	+
85	♂	—	—	—	144	♂	—	—	—
86	♂	—	—	+	145	♂	—	—	+
87	♂	—	—	—	146	♂	—	—	—
88	♂	—	—	—	147	♂	—	+	+
89	♂	—	—	—	148	♂	+	—	+
90	♂	—	—	+	149	♂	+	—	+
91	♂	+	+	+	150	♂	—	—	+
92	♂	+	—	—	151	♂	—	—	+
93	♂	—	—	—	152	♂	—	—	+
94	♂	—	—	+	153	♂	+	—	+
95	♂	—	—	+	154	♂	—	—	+
96	♂	—	—	+	155	♂	—	—	—
97	♂	—	+	+	156	♂	—	—	—
98	♂	—	+	+	157	♂	—	+	+
99	♂	—	—	—	158	♂	—	—	—
100	♂	—	—	+	159	♂	—	—	+
101	♂	+	—	+	160	♂	—	—	—
102	♂	+	—	+	161	♂	+	—	+
103	♂	—	—	—	162	♂	—	—	—
104	♂	+	—	—	163	♂	—	—	+
105	♂	—	—	—	164	♂	—	—	—
106	♂	+	—	+	165	♂	—	—	—
107	♂	—	—	—	166	♂	—	—	—
108	♂	—	—	+	167	♂	+	—	+
109	♂	—	—	—	168	♂	+	—	+
110	♂	—	—	—	169	♂	—	+	—
111	♂	+	—	—	170	♂	+	—	+
112	♂	—	—	+	171	♂	—	—	+
113	♂	—	—	+	172	♂	—	—	+
114	♂	—	—	+	173	♂	+	—	+
115	♂	—	—	—	174	♂	—	—	—
116	♂	—	+	+	175	♂	—	—	+
117	♂	—	—	+	176	♂	—	+	+
118	♂	—	—	—	177	♂	+	+	+
119	♂	+	—	+	178	♂	+	—	—
120	♂	—	+	+	179	♂	+	—	—
121	♂	—	+	+	180	♂	+	—	+
122	♂	—	—	—	181	♂	—	—	+
123	♂	—	+	+	182	♂	—	—	—
124	♂	—	—	—	183	♂	—	—	—
125	♂	—	—	—					

In the present experiment, the observer landed by canoe and made the paddlers sit round him; the fly-boys moved about amongst them and caught the flies as they came to feed. They were handed at once to the observer, who snipped off their heads, noted their sex, and roughly dissected out the alimentary canal, and, when it contained food-stuff, smeared it at full length on a slide. Notes were made as to the naked-eye contents of the canal and the question of pregnancy. The smears were fixed in the usual way and brought to the laboratory, where they were stained and examined minutely.

The total number of flies examined was 183, of which 104 (57 per cent.) were males and 79 (43 per cent.) were females; of the total number, 108 (59 per cent.) contained blood in a more or less digested state, and 75 (41 per cent.) contained no food-stuff. Out of the 79 females, 32 (40 per cent.) contained nearly fully-developed larvæ.

Table III.—Shows the Nature of the Blood in the Interior of the Flies, and also the Number of Flies which contained Parasites.

No.	Mammalian blood.	Non-mammalian blood.	Trypanosomes.	Halteridia.	No.	Mammalian blood.	Non-mammalian blood.	Trypanosomes.	Halteridia.
1	—	+	—	—	27	—	+	—	—
2	—	+	—	+	28	—	+	—	—
3	—	+	+	—	29	—	+	—	—
4	—	+	—	—	30	—	+	+	—
5	—	+	—	—	31	—	+	—	—
6	—	+	—	—	32	+	—	—	—
7	—	+	—	—	33	—	+	+	—
8	—	+	—	—	34	—	+	—	—
9	—	+	—	—	35	—	+	—	—
10	—	—	+	—	36	—	—	+	—
11	+	—	—	—	37	—	+	—	—
12	—	+	—	—	38	—	—	+	—
13	—	—	+	—	39	—	+	—	—
14	—	+	—	+	40	+	—	—	—
15	—	—	+	—	41	—	—	+	—
16	—	+	—	—	42	—	+	—	—
17	—	+	—	+	43	—	+	—	—
18	—	—	+	—	44	—	+	—	+
19	—	+	—	—	45	—	+	—	—
20	—	—	+	—	46	+	—	—	—
21	+	+	—	—	47	—	+	—	—
22	—	+	—	—	48	—	—	+	—
23	—	+	—	—	49	—	—	+	—
24	—	+	Trypano-plasma	—	50	—	+	—	—
25	—	+	—	+	51	+	—	—	—
26	—	—	+	—	52	+	—	—	—

The above table shows that out of the original 183 flies examined, 52 (28 per cent.) contained blood which was recent enough to show the red corpuscles. Out of these 52, 7 (13 per cent.) were from mammalian blood, whilst non-mammalian blood was present in 35 flies (67 per cent.). Trypanosomes were present in 14 flies (27 per cent.) and Halteridia in 5 (nearly 10 per cent.). One contained a trypanoplasma, derived, probably, from bird's blood.

An attempt was made to separate the nucleated red blood corpuscles into avian and reptilian. The distinction was made by size only, it being found from the measurement of corpuscles from birds and reptiles that the average normal length was:—

Standard amphibian (crocodile), 15·4 microns.

Standard avian (horn-bill), 13·1 microns.

In examining the smears from the flies, the average was taken of 10 to 20 red cells, which seemed as natural as possible, and the average obtained in

Table IV.—Shows the Average Measurements of the Nucleated Red Corpuscles and their Probable Origin.

No.	Average length of red cells, in microns.	Probably reptilian or amphibian.	Probably avian.
1	15·3	+	—
2	14·2	+	—
3	14·1	+	—
4	13·3	—	+
5	14·4	+	—
6	14·0	+	—
7	14·0	+	—
8	14·4	+	—
9	14·5	+	—
10	13·0	—	+
11	14·8	+	—
12	14·4	+	—
13	10·6	—	+
14	13·4	—	+
15	14·6	+	—
16	13·2	—	+
17	14·0	+	—
18	15·2	+	—
19	13·2	—	+
20	13·3	—	+
Totals.....		13	7

*Remarks.*—Only 20 of the flies contained nucleated blood which was recent enough to justify any deduction as to its origin. Out of these 20 flies, 13 had probably fed on a reptile or amphibian, and 7 on a bird.

this way compared with the standard measurements. No allowance could be made for alterations due to digestive changes: these were presumed to be similar in all cases.

Table V.—Showing the Result of the Examination of *Glossina palpalis* from "Crocodile Point."

No. of flies.	♂.	♀.	♀ containing larvae.	Intestine empty.	Intestine containing blood.	Mammalian blood.	Non-mammalian blood.	Non-mammalian blood.		Flagellates.	Halteridia.
								Avian.	Reptilian.		
183	104	79	32	75	108	7	35	7	13	14	5
Percentages...	57	43	17·4	41	59	3·8	19·1	3·8	7	7·6	2·7

#### *Conclusions.*

Two hundred and twenty *Glossinæ palpalis* were caught on various parts of the Lake-shore, and at intervals extending over several months; they were examined about 24 hours after capture. The examination of their intestinal contents revealed the fact that about 27 per cent. contained the remains of blood, the majority of which was of mammalian origin.

In the second experiment, 183 *Glossinæ palpalis* were caught at one spot where the food supply was abundant—birds and crocodiles—and the flies were examined at once. A much higher percentage (nearly 60 per cent.) contained the remains of a blood meal. The blood in the majority of the flies had been obtained from birds or reptiles, and of these the reptilian blood was twice as frequent as the blood of birds.